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Title: NUCLEOTIDE SEQUENCES ENCODING A SENSOR KINASE, *citA*, FROM  
CORYNEBACTERIUM GLUTAMICUM


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The undersigned, of the below address, hereby states that he/she well knows both the English and German languages, and that the attached is an accurate translation into the English language of the Certified Copy, filed for this application under 35 U.S.C. Section 119 and/or 365, of:

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Respectfully submitted,  
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Neue für das citA-Gen kodierende Nukleotidsequenzen

identified by the code number 000169 BT at the upper left of each page and corresponding to client/matter number of the law firm of

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Date: 20<sup>th</sup> March 2003

By: *Janet Hope*

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## Certificate of Priority for Filing of a Patent Application

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Applicant/Proprietor: Degussa AG, Düsseldorf/Germany  
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Title: New nucleotide sequences which code  
for the citA gene

IPC: C 12 N, C 07 K, C 07 H

The attached papers are a true and accurate reproduction of  
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Munich, 21st June 2001

On behalf of the President  
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**New nucleotide sequences which code for the citA gene**

The invention provides nucleotide sequences from coryneform bacteria which code for the citA gene and a process for the fermentative preparation of amino acids, in particular L-lysine, by attenuation of the citA gene. The citA gene codes for the sensor kinase Cit A of a two-component system.

**Prior art**

L-Amino acids, in particular L-lysine, are used in human medicine and in the pharmaceuticals industry, in the foodstuffs industry and very particularly in animal nutrition.

It is known that amino acids are prepared by fermentation from strains of coryneform bacteria, in particular Corynebacterium glutamicum. Because of their great importance, work is constantly being undertaken to improve the preparation processes. Improvements to the process can relate to fermentation measures, such as, for example, stirring and supply of oxygen, or the composition of the nutrient media, such as, for example, the sugar concentration during the fermentation, or the working up to the product form by, for example, ion exchange chromatography, or the intrinsic output properties of the microorganism itself.

Methods of mutagenesis, selection and mutant selection are used to improve the output properties of these microorganisms. Strains which are resistant to antimetabolites or are auxotrophic for metabolites of regulatory importance and which produce amino acids are obtained in this manner.

Methods of the recombinant DNA technique have also been employed for some years for improving the strain of *Corynebacterium* strains which produce L-amino acids.

Object of the invention

- 5 The inventors had the object of providing new measures for improved fermentative preparation of amino acids, in particular L-lysine.

Description of the invention

- If L-lysine or lysine are mentioned in the following, this  
10 also means the salts, such as e. g. lysine monohydrochloride or lysine sulfate.

The invention provides an isolated polynucleotide from coryneform bacteria, comprising a polynucleotide sequence which codes for the *citA* gene chosen from the group  
15 consisting of

- a) polynucleotide which is identical to the extent of at least 70 % to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 2,
- 20 b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70 % to the amino acid sequence of SEQ ID No. 2,
- c) polynucleotide which is complementary to the  
25 polynucleotides of a) or b), and
- d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequence of a), b) or c),

the polypeptide preferably having the activity of sensor  
30 kinase CitA.

The invention also provides the abovementioned polynucleotide, this preferably being a DNA which is capable of replication, comprising:

- (i) the nucleotide sequence shown in SEQ ID No.1 or
- 5 (ii) at least one sequence which corresponds to sequence (i) within the range of the degeneration of the genetic code, or
- (iii) at least one sequence which hybridizes with the sequences complementary to sequences (i) or (ii),  
10 and optionally
- (iv) sense mutations of neutral function in (i).

The invention also provides:

a polynucleotide, in particular DNA, which is capable of replication and comprises the nucleotide sequence as  
15 shown in SEQ ID No.1;

a polynucleotide which codes for a polypeptide which comprises the amino acid sequence as shown in SEQ ID No. 2;

a vector containing parts of the polynucleotide according  
20 to the invention, but at least 15 successive nucleotides of the sequence claimed

and coryneform bacteria in which the citA gene is attenuated, in particular by an insertion or deletion.

The invention also provides polynucleotides which  
25 substantially comprise a polynucleotide sequence, which are obtainable by screening by means of hybridization of a corresponding gene library of a coryneform bacterium, which comprises the complete gene or parts thereof, with a probe which comprises the sequence of the polynucleotide

according to the invention or a fragment thereof, and isolation of the polynucleotide sequence mentioned.

Polynucleotides comprising the sequences according to the invention are suitable as hybridization probes for RNA,  
5 cDNA and DNA, in order to isolate, in the full length, nucleic acids or polynucleotides or genes which code for CitA protein or to isolate those nucleic acids or polynucleotides or genes which have a high similarity with the sequence of the citA gene.

10 Polynucleotides comprising the sequences according to the invention are furthermore suitable as primers with the aid of which DNA of genes which code for the CitA protein can be prepared by the polymerase chain reaction (PCR).

Such oligonucleotides which serve as probes or primers  
15 comprise at least 30, preferably at least 20, very particularly preferably at least 15 successive nucleotides. Oligonucleotides which have a length of at least 40 or 50 nucleotides are also suitable.

"Isolated" means separated out of its natural environment.

20 "Polynucleotide" in general relates to polyribonucleotides and polydeoxyribonucleotides, it being possible for these to be non-modified RNA or DNA or modified RNA or DNA.

The polynucleotides according to the invention include a polynucleotide according to SEQ ID No. 1 or a fragment  
25 prepared therefrom and also those which are at least 70 %, preferably at least 80 % and in particular at least 90 % to 95 % identical to the polynucleotide according to SEQ ID No. 1 or a fragment prepared therefrom.

"Polypeptides" are understood as meaning peptides or  
30 proteins which comprise two or more amino acids bonded via peptide bonds.

The polypeptides according to the invention include a polypeptide according to SEQ ID No. 2, in particular those with the biological activity of the CitA protein and also those which are at least 70 %, preferably at least 80 % and  
5 in particular at least 90 % to 95 % identical to the polypeptide according to SEQ ID No. 2 and have the activity mentioned.

The invention moreover relates to a process for the fermentative preparation of amino acids, in particular L-  
10 lysine, using coryneform bacteria which in particular already produce amino acids and in which the nucleotide sequences which code for the citA gene are attenuated, in particular eliminated or expressed at a low level.

The term "attenuation" in this connection describes the  
15 reduction or elimination of the intracellular activity of one or more enzymes (proteins) in a microorganism which are coded by the corresponding DNA, for example by using a weak promoter or using a gene or allele which codes for a corresponding enzyme with a low activity or inactivates the  
20 corresponding gene or enzyme (protein), and optionally combining these measures.

The microorganisms which the present invention provides can prepare amino acids, in particular L-lysine, from glucose, sucrose, lactose, fructose, maltose, molasses, starch,  
25 cellulose or from glycerol and ethanol. They can be representatives of coryneform bacteria, in particular of the genus *Corynebacterium*. Of the genus *Corynebacterium*, there may be mentioned in particular the species *Corynebacterium glutamicum*, which is known among experts  
30 for its ability to produce L-amino acids.

Suitable strains of the genus *Corynebacterium*, in particular of the species *Corynebacterium glutamicum* (*C. glutamicum*), are in particular the known wild-type strains



- Corynebacterium glutamicum ATCC13032  
Corynebacterium acetoglutamicum ATCC15806  
Corynebacterium acetoacidophilum ATCC13870  
Corynebacterium melassecola ATCC17965  
5 Corynebacterium thermoaminogenes FERM BP-1539  
Brevibacterium flavum ATCC14067  
Brevibacterium lactofermentum ATCC13869 and  
Brevibacterium divaricatum ATCC14020

or L-amino acid-producing mutants or strains prepared  
10 therefrom, such as, for example, the L-lysine-producing  
strains

- Corynebacterium glutamicum FERM-P 1709  
Brevibacterium flavum FERM-P 1708  
Brevibacterium lactofermentum FERM-P 1712  
15 Corynebacterium glutamicum FERM-P 6463  
Corynebacterium glutamicum FERM-P 6464  
Corynebacterium glutamicum DM58-1  
Corynebacterium glutamicum DG52-5  
Corynebacterium glutamicum DSM 5715 and  
20 Corynebacterium glutamicum DSM 12866

The inventors have succeeded in isolating the new *citA* gene  
of *C. glutamicum* which codes for the Cita protein and which  
is a sensor kinase of a two-component system.

To isolate the *citA* gene or also other genes of *C.*  
25 *glutamicum*, a gene library of this microorganism is first  
set up in *Escherichia coli* (*E. coli*). The setting up of  
gene libraries is described in generally known textbooks  
and handbooks. The textbook by Winnacker: *Gene und Klone, Eine Einführung in die Gentechnologie* [Genes and Clones, An  
30 Introduction to Genetic Engineering] (Verlag Chemie,  
Weinheim, Germany, 1990), or the handbook by Sambrook et  
al.: *Molecular Cloning, A Laboratory Manual* (Cold Spring  
Harbor Laboratory Press, 1989) may be mentioned as an  
example. A well-known gene library is that of the *E. coli*

K-12 strain W3110 set up in  $\lambda$ -vectors by Kohara et al. (Cell 50, 495-508 (1987)). Bathe et al. (Molecular and General Genetics, 252:255-265, 1996) describe a gene library of *C. glutamicum* ATCC13032, which was set up with the aid of the cosmid vector SuperCos I (Wahl et al., 1987, Proceedings of the National Academy of Sciences USA, 84:2160-2164) in the *E. coli* K-12 strain NM554 (Raleigh et al., 1988, Nucleic Acids Research 16:1563-1575). Börmann et al. (Molecular Microbiology 6(3), 317-326 (1992)) in turn describe a gene library of *C. glutamicum* ATCC13032 using the cosmid pH79 (Hohn and Collins, 1980, Gene 11, 291-298).

To prepare a gene library of *C. glutamicum* in *E. coli* it is also possible to use plasmids such as pBR322 (Bolivar, 1979, Life Sciences, 25, 807-818) or pUC9 (Vieira et al., 1982, Gene, 19:259-268). Suitable hosts are, in particular, those *E. coli* strains which are restriction- and recombination-defective, such as, for example, the strain DH5 $\alpha$  (Jeffrey H. Miller: "A Short Course in Bacterial Genetics, A Laboratory Manual and Handbook for *Escherichia coli* and Related Bacteria", Cold Spring Harbor Laboratory Press, 1992).

The long DNA fragments cloned with the aid of cosmids or other  $\lambda$ -vectors can then be subcloned in turn into the usual vectors suitable for DNA sequencing.

Methods of DNA sequencing are described, inter alia, by Sanger et al. (Proceedings of the National Academy of Sciences of the United States of America USA, 74:5463-5467, 1977).

The resulting DNA sequences can then be investigated with known algorithms or sequence analysis programs, such as e.g. that of Staden (Nucleic Acids Research 14, 217-232(1986)), that of Marck (Nucleic Acids Research 16,

1829-1836 (1988)) or the GCG program of Butler (Methods of Biochemical Analysis 39, 74-97 (1998)).

The new DNA sequence of *C. glutamicum* which codes for the *citA* gene and which, as SEQ ID No. 1, is a constituent of  
5 the present invention has been found in this way. The amino acid sequence of the corresponding protein has furthermore been derived from the present DNA sequence by the methods described above. The resulting amino acid sequence of the *citA* gene product is shown in SEQ ID No. 2.

10 Coding DNA sequences which result from SEQ ID No. 1 by the degeneracy of the genetic code are also a constituent of the invention. In the same way, DNA sequences which hybridize with SEQ ID No. 1 or parts of SEQ ID No. 1 are a constituent of the invention. Conservative amino acid  
15 exchanges, such as e.g. exchange of glycine for alanine or of aspartic acid for glutamic acid in proteins, are furthermore known among experts as "sense mutations" which do not lead to a fundamental change in the activity of the protein, i.e. are of neutral function. It is furthermore  
20 known that changes on the N and/or C terminus of a protein cannot substantially impair or can even stabilize the function thereof. Information in this context can be found by the expert, inter alia, in Ben-Bassat et al. (Journal of Bacteriology 169:751-757 (1987)), in O'Regan et al. (Gene  
25 77:237-251 (1989)), in Sahin-Toth et al. (Protein Sciences 3:240-247 (1994)), in Hochuli et al. (Bio/Technology 6:1321-1325 (1988)) and in known textbooks of genetics and molecular biology. Amino acid sequences which result in a corresponding manner from SEQ ID No. 2 are also a  
30 constituent of the invention.

Finally, DNA sequences which are prepared by the polymerase chain reaction (PCR) using primers which result from SEQ ID No. 1 are a constituent of the invention. Such  
oligonucleotides typically have a length of at least 15  
35 nucleotides.

Instructions for identifying DNA sequences by means of hybridization can be found by the expert, inter alia, in the handbook "The DIG System Users Guide for Filter Hybridization" from Boehringer Mannheim GmbH (Mannheim, Germany, 1993) and in Liebl et al. (International Journal of Systematic Bacteriology 41: 255-260 (1991)). The hybridization takes place under stringent conditions, that is to say only hybrids in which the probe and target sequence, i. e. the polynucleotides treated with the probe, are at least 70 % identical are formed. It is known that the stringency of the hybridization, including the washing steps, is influenced or determined by varying the buffer composition, the temperature and the salt concentration. The hybridization reaction is preferably carried out under a relatively low stringency compared with the washing steps (Hybaid Hybridisation Guide, Hybaid Limited, Teddington, UK, 1996). A 5x SSC buffer at a temperature of approx. 50 - 68°C, for example, can be employed for the hybridization reaction. Probes can also hybridize here with polynucleotides which are less than 70 % identical to the sequence of the probe. Such hybrids are less stable and are removed by washing under stringent conditions. This can be achieved, for example, by lowering the salt concentration to 2x SSC and optionally subsequently 0.5x SSC (The DIG System User's Guide for Filter Hybridisation, Boehringer Mannheim, Mannheim, Germany, 1995) a temperature of approx. 50 - 68°C being established. Polynucleotide fragments which are, for example, at least 70 % or at least 80 % or at least 90 % to 95 % identical to the sequence of the probe employed can be isolated by increasing the hybridization temperature stepwise from 50 to 68°C in steps of approx. 1 - 2°C. Further instructions on hybridization are obtainable on the market in the form of so-called kits (e.g. DIG Easy Hyb from Roche Diagnostics GmbH, Mannheim, Germany, Catalogue No. 1603558).

Instructions for amplification of DNA sequences with the aid of the polymerase chain reaction (PCR) can be found by the expert, inter alia, in the handbook by Gait: Oligonucleotides: A Practical Approach (IRL Press, Oxford, UK, 1984) and in Newton and Graham: PCR (Spektrum Akademischer Verlag, Heidelberg, Germany, 1994).

In the work on the present invention, it has been found that coryneform bacteria produce amino acids, in particular L-lysine, in an improved manner after attenuation of the citA gene.

To achieve an attenuation, either the expression of the citA gene or the catalytic properties of the enzyme protein can be reduced or eliminated. The two measures can optionally be combined.

The reduction in gene expression can take place by suitable culturing or by genetic modification (mutation) of the signal structures of gene expression. Signal structures of gene expression are, for example, repressor genes, activator genes, operators, promoters, attenuators, ribosome binding sites, the start codon and terminators. The expert can find information on this e.g. in the patent application WO 96/15246, in Boyd and Murphy (Journal of Bacteriology 170: 5949 (1988)), in Voskuil and Chambliss (Nucleic Acids Research 26: 3548 (1998)), in Jensen and Hammer (Biotechnology and Bioengineering 58: 191 (1998)), in Pátek et al. (Microbiology 142: 1297 (1996)), Vasicova et al. (Journal of Bacteriology 181: 6188 (1999)) and in known textbooks of genetics and molecular biology, such as e.g. the textbook by Knippers ("Molekulare Genetik [Molecular Genetics]", 6th edition, Georg Thieme Verlag, Stuttgart, Germany, 1995) or that by Winnacker ("Gene und Klone [Genes and Clones]", VCH Verlagsgesellschaft, Weinheim, Germany, 1990).

Mutations which lead to a change or reduction in the catalytic properties of enzyme proteins are known from the prior art; examples which may be mentioned are the works by Qiu and Goodman (Journal of Biological Chemistry 272: 8611-8617 (1997)), Sugimoto et al. (Bioscience Biotechnology and Biochemistry 61: 1760-1762 (1997)) and Möckel ("Die Threonindehydratase aus Corynebacterium glutamicum: Aufhebung der allosterischen Regulation und Struktur des Enzyms [Threonine dehydratase from Corynebacterium glutamicum: Cancelling the allosteric regulation and structure of the enzyme]", Reports from the Jülich Research Centre, Jül-2906, ISSN09442952, Jülich, Germany, 1994). Summarizing descriptions can be found in known textbooks of genetics and molecular biology, such as e.g. that by Hagemann ("Allgemeine Genetik [General Genetics]", Gustav Fischer Verlag, Stuttgart, 1986).

Possible mutations are transitions, transversions, insertions and deletions. Depending on the effect of the amino acid exchange on the enzyme activity, missense mutations or nonsense mutations are referred to. Insertions or deletions of at least one base pair (bp) in a gene lead to frame shift mutations, as a consequence of which incorrect amino acids are incorporated or translation is interrupted prematurely. Deletions of several codons typically lead to a complete loss of the enzyme activity. Instructions on generation of such mutations are prior art and can be found in known textbooks of genetics and molecular biology, such as e.g. the textbook by Knippers ("Molekulare Genetik [Molecular Genetics]", 6th edition, Georg Thieme Verlag, Stuttgart, Germany, 1995), that by Winnacker ("Gene und Klone [Genes and Clones]", VCH Verlagsgesellschaft, Weinheim, Germany, 1990) or that by Hagemann ("Allgemeine Genetik [General Genetics]", Gustav Fischer Verlag, Stuttgart, 1986).

A common method of mutating genes of *C. glutamicum* is the method of gene disruption and gene replacement described by Schwarzer and Pühler (Bio/Technology 9, 84-87 (1991)).

In the method of gene disruption a central part of the  
5 coding region of the gene of interest is cloned in a  
plasmid vector which can replicate in a host (typically *E. coli*), but not in *C. glutamicum*. Possible vectors are, for  
example, pSUP301 (Simon et al., Bio/Technology 1, 784-791  
(1983)), pK18mob or pK19mob (Schäfer et al., Gene 145, 69-  
10 73 (1994)), pK18mobsacB or pK19mobsacB (Jäger et al.,  
Journal of Bacteriology 174: 5462-65 (1992)), pGEM-T  
(Promega corporation, Madison, WI, USA), pCR2.1-TOPO  
(Shuman (1994). Journal of Biological Chemistry 269:32678-  
84; US Patent 5,487,993), pCR®Blunt (Invitrogen,  
15 Groningen, Holland; Bernard et al., Journal of Molecular  
Biology, 234: 534-541 (1993)) or pEM1 (Schrumpf et al,  
1991, Journal of Bacteriology 173:4510-4516). The plasmid  
vector which contains the central part of the coding region  
of the gene is then transferred into the desired strain of  
20 *C. glutamicum* by conjugation or transformation. The method  
of conjugation is described, for example, by Schäfer et al.  
(Applied and Environmental Microbiology 60, 756-759  
(1994)). Methods for transformation are described, for  
example, by Thierbach et al. (Applied Microbiology and  
25 Biotechnology 29, 356-362 (1988)), Dunican and Shivnan  
(Bio/Technology 7, 1067-1070 (1989)) and Tauch et al. (FEMS  
Microbiological Letters 123, 343-347 (1994)). After  
homologous recombination by means of a "cross-over" event,  
the coding region of the gene in question is interrupted by  
30 the vector sequence and two incomplete alleles are  
obtained, one lacking the 3' end and one lacking the 5'  
end. This method has been used, for example, by Fitzpatrick  
et al. (Applied Microbiology and Biotechnology 42, 575-580  
(1994)) to eliminate the *recA* gene of *C. glutamicum*.

In the method of gene replacement, a mutation, such as e.g. a deletion, insertion or base exchange, is established in vitro in the gene of interest. The allele prepared is in turn cloned in a vector which is not replicative for *C. glutamicum* and this is then transferred into the desired host of *C. glutamicum* by transformation or conjugation. After homologous recombination by means of a first "cross-over" event which effects integration and a suitable second "cross-over" event which effects excision in the target gene or in the target sequence, the incorporation of the mutation or of the allele is achieved. This method was used, for example, by Peters-Wendisch et al. (Microbiology 144, 915 - 927 (1998)) to eliminate the *pyc* gene of *C. glutamicum* by a deletion.

15 A deletion, insertion or a base exchange can be incorporated into the *citA* gene in this manner.

In addition, it may be advantageous for the production of L-amino acids, in particular L-lysine, to enhance, in particular to over-express, one or more enzymes of the particular biosynthesis pathway, of glycolysis, of anaplerosis, of the pentose phosphate cycle or of amino acid export and optionally regulatory proteins, in addition to attenuation of the *citA* gene.

Thus, for example, for the preparation of L-lysine, at the same time one or more of the genes chosen from the group consisting of

- the *dapA* gene which codes for dihydrodipicolinate synthase (EP-B 0 197 335),
- the *gap* gene which codes for glyceraldehyde 3-phosphate dehydrogenase (Eikmanns (1992), Journal of Bacteriology 174:6076-6086),
- the *zwf* gene which codes for glucose 6-phosphate dehydrogenase (JP-A-09224661),



- the pyc gene which codes for pyruvate carboxylase (DE-A-198 31 609),
  - the lysE gene which codes for lysine export (DE-A-195 48 222)
- 5 • the lysC gene which codes for a feed back resistant aspartate kinase (Kalinowski et al. (1990), Molecular and General Genetics 224, 317-324; Accession No. P26512), or
- the zwal gene which codes for the Zwal protein (DE: 199 59 328.0, DSM 13115)
- 10 can be enhanced, in particular over-expressed.

It may furthermore be advantageous for the production of amino acids, in particular L-lysine, in addition to the attenuation of the citA gene, at the same time for one or more of the genes chosen from the group consisting of

- 15 • the pck gene which codes for phosphoenol pyruvate carboxykinase (DE 199 50 409.1, DSM 13047),
- the pgi gene which codes for glucose 6-phosphate isomerase (US 09/396,478, DSM 12969),
  - the poxB gene which codes for pyruvate oxidase
- 20 (DE:1995 1975.7, DSM 13114)
- the zwa2 gene which codes for the Zwa2 protein (DE: 199 59,327.2, DSM 13113)

to be attenuated.

- In addition to attenuation of the citA gene it may
- 25 furthermore be advantageous, for the production of amino acids, in particular L-lysine, to eliminate undesirable side reactions, (Nakayama: "Breeding of Amino Acid Producing Microorganisms", in: Overproduction of Microbial

Products, Krumphanzl, Sikyta, Vanek (eds.), Academic Press, London, UK, 1982).

The invention also provides the microorganisms prepared according to the invention, and these can be cultured  
5 continuously or discontinuously in the batch process (batch culture) or in the fed batch (feed process) or repeated fed batch process (repetitive feed process) for the purpose of production of L-amino acids, in particular L-lysine. A summary of known culture methods is described in the  
10 textbook by Chmiel (Bioprozesstechnik 1. Einführung in die Bioverfahrenstechnik [Bioprocess Technology 1. Introduction to Bioprocess Technology (Gustav Fischer Verlag, Stuttgart, 1991)) or in the textbook by Storhas (Bioreaktoren und periphere Einrichtungen [Bioreactors and Peripheral  
15 Equipment] (Vieweg Verlag, Braunschweig/Wiesbaden, 1994)).

The culture medium to be used must meet the requirements of the particular strains in a suitable manner. Descriptions of culture media for various microorganisms are contained in the handbook "Manual of Methods for General  
20 Bacteriology" of the American Society for Bacteriology (Washington D.C., USA, 1981). Sugars and carbohydrates, such as e.g. glucose, sucrose, lactose, fructose, maltose, molasses, starch and cellulose, oils and fats, such as, for example, soya oil, sunflower oil, groundnut oil and coconut  
25 fat, fatty acids, such as, for example, palmitic acid, stearic acid and linoleic acid, alcohols, such as, for example, glycerol and ethanol, and organic acids, such as, for example, acetic acid, can be used as the source of carbon. These substances can be used individually or as a  
30 mixture.

Organic nitrogen-containing compounds, such as peptones, yeast extract, meat extract, malt extract, corn steep liquor, soya bean flour and urea, or inorganic compounds, such as ammonium sulfate, ammonium chloride, ammonium  
35 phosphate, ammonium carbonate and ammonium nitrate, can be

used as the source of nitrogen. The sources of nitrogen can be used individually or as a mixture.

Phosphoric acid, potassium dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium-  
5 containing salts can be used as the source of phosphorus. The culture medium must furthermore comprise salts of metals, such as, for example, magnesium sulfate or iron sulfate, which are necessary for growth. Finally, essential growth substances, such as amino acids and vitamins, can be  
10 employed in addition to the abovementioned substances. Suitable precursors can moreover be added to the culture medium. The starting substances mentioned can be added to the culture in the form of a single batch, or can be fed in during the culture in a suitable manner.

15 Basic compounds, such as sodium hydroxide, potassium hydroxide, ammonia or aqueous ammonia, or acid compounds, such as phosphoric acid or sulfuric acid, can be employed in a suitable manner to control the pH of the culture. Antifoams, such as, for example, fatty acid polyglycol  
20 esters, can be employed to control the development of foam. Suitable substances having a selective action, such as, for example, antibiotics, can be added to the medium to maintain the stability of plasmids. To maintain aerobic conditions, oxygen or oxygen-containing gas mixtures, such  
25 as, for example, air, are introduced into the culture. The temperature of the culture is usually 20°C to 45°C, and preferably 25°C to 40°C. Culturing is continued until a maximum of the desired product has formed. This target is usually reached within 10 hours to 160 hours.

30 Methods for the determination of L-amino acids are known from the prior art. The analysis can thus be carried out, for example, as described by Spackman et al. (Analytical Chemistry, 30, (1958), 1190) by anion exchange chromatography with subsequent ninhydrin derivatization, or  
35 it can be carried out by reversed phase HPLC, for example

as described by Lindroth et al. (Analytical Chemistry (1979) 51: 1167-1174).

The invention furthermore relates to a process for the fermentative preparation of an amino acid chosen from the group consisting of L-asparagine, L-threonine, L-serine, L-glutamate, L-glycine, L-alanine, L-cysteine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-lysine, L-tryptophan and L-arginine, in particular L-lysine, using coryneform bacteria which in particular already produce one or more of the amino acids mentioned.

The present invention is explained in more detail in the following with the aid of embodiment examples.

The isolation of plasmid DNA from Escherichia coli and all techniques of restriction, Klenow and alkaline phosphatase treatment were carried out by the method of Sambrook et al. (Molecular Cloning. A Laboratory Manual, 1989, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA). Methods for transformation of Escherichia coli are also described in this handbook.

The composition of the usual nutrient media, such as LB or TY medium, can also be found in the handbook by Sambrook et al.

ExamplesExample 1

Preparation of a genomic cosmid gene library from *C. glutamicum* ATCC 13032

- 5 Chromosomal DNA from *C. glutamicum* ATCC 13032 was isolated as described by Tauch et al. (1995, Plasmid 33:168-179) and partly cleaved with the restriction enzyme Sau3AI (Amersham Pharmacia, Freiburg, Germany, Product Description Sau3AI, Code no. 27-0913-02). The DNA fragments were
- 10 dephosphorylated with shrimp alkaline phosphatase (Roche Molecular Biochemicals, Mannheim, Germany, Product Description SAP, Code no. 1758250). The DNA of the cosmid vector SuperCos1 (Wahl et al. (1987), Proceedings of the National Academy of Sciences, USA 84:2160-2164), obtained
- 15 from Stratagene (La Jolla, USA, Product Description SuperCos1 Cosmid Vektor Kit, Code no. 251301) was cleaved with the restriction enzyme XbaI (Amersham Pharmacia, Freiburg, Germany, Product Description XbaI, Code no. 27-0948-02) and likewise dephosphorylated with shrimp alkaline
- 20 phosphatase.

- The cosmid DNA was then cleaved with the restriction enzyme BamHI (Amersham Pharmacia, Freiburg, Germany, Product Description BamHI, Code no. 27-0868-04). The cosmid DNA treated in this manner was mixed with the treated ATCC13032
- 25 DNA and the batch was treated with T4 DNA ligase (Amersham Pharmacia, Freiburg, Germany, Product Description T4-DNA-Ligase, Code no.27-0870-04). The ligation mixture was then packed in phages with the aid of Gigapack II XL Packing Extract (Stratagene, La Jolla, USA, Product Description
- 30 Gigapack II XL Packing Extract, Code no. 200217).

For infection of the *E. coli* strain NM554 (Raleigh et al. 1988, Nucleic Acid Res. 16:1563-1575) the cells were taken up in 10 mM MgSO<sub>4</sub> and mixed with an aliquot of the phage

suspension. The infection and titering of the cosmid library were carried out as described by Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor), the cells being plated out on LB agar (Lennox, 5 1955, Virology, 1:190) + 100 µg/ml ampicillin. After incubation overnight at 37°C, recombinant individual clones were selected.

### Example 2

#### Isolation and sequencing of the citA gene

10 The cosmid DNA of an individual colony was isolated with the Qiaprep Spin Miniprep Kit (Product No. 27106, Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions and partly cleaved with the restriction enzyme Sau3AI (Amersham Pharmacia, Freiburg, Germany, Product 15 Description Sau3AI, Product No. 27-0913-02). The DNA fragments were dephosphorylated with shrimp alkaline phosphatase (Roche Molecular Biochemicals, Mannheim, Germany, Product Description SAP, Product No. 1758250). After separation by gel electrophoresis, the cosmid 20 fragments in the size range of 1500 to 2000 bp were isolated with the QiaExII Gel Extraction Kit (Product No. 20021, Qiagen, Hilden, Germany).

The DNA of the sequencing vector pZero-1, obtained from Invitrogen (Groningen, The Netherlands, Product Description 25 Zero Background Cloning Kit, Product No. K2500-01) was cleaved with the restriction enzyme BamHI (Amersham Pharmacia, Freiburg, Germany, Product Description BamHI, Product No. 27-0868-04). The ligation of the cosmid fragments in the sequencing vector pZero-1 was carried out 30 as described by Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor), the DNA mixture being incubated overnight with T4 ligase (Pharmacia Biotech, Freiburg, Germany). This ligation mixture was then electroporated (Tauch et al. 1994, FEMS Microbiol Letters,

123:343-7) into the E. coli strain DH5 $\alpha$ MCR (Grant, 1990, Proceedings of the National Academy of Sciences, U.S.A., 87:4645-4649) and plated out on LB agar (Lennox, 1955, Virology, 1:190) with 50  $\mu$ g/ml zeocin.

- 5 The plasmid preparation of the recombinant clones was carried out with Biorobot 9600 (Product No. 900200, Qiagen, Hilden, Germany). The sequencing was carried out by the dideoxy chain termination method of Sanger et al. (1977, Proceedings of the National Academies of Sciences, U.S.A.,  
10 74:5463-5467) with modifications according to Zimmermann et al. (1990, Nucleic Acids Research, 18:1067). The "RR dRhodamin Terminator Cycle Sequencing Kit" from PE Applied Biosystems (Product No. 403044, Weiterstadt, Germany) was used. The separation by gel electrophoresis and analysis of  
15 the sequencing reaction were carried out in a "Rotiphoresis NF Acrylamide/Bisacrylamide" Gel (29:1) (Product No. A124.1, Roth, Karlsruhe, Germany) with the "ABI Prism 377" sequencer from PE Applied Biosystems (Weiterstadt, Germany).
- 20 The raw sequence data obtained were then processed using the Staden program package (1986, Nucleic Acids Research, 14:217-231) version 97-0. The individual sequences of the pZerol derivatives were assembled to a continuous contig. The computer-assisted coding region analyses were prepared  
25 with the XNIP program (Staden, 1986, Nucleic Acids Research, 14:217-231). Further analyses were carried out with the "BLAST search program" (Altschul et al., 1997, Nucleic Acids Research, 25:3389-3402) against the non-redundant databank of the "National Center for  
30 Biotechnology Information" (NCBI, Bethesda, MD, USA).

The resulting nucleotide sequence is shown in SEQ ID No. 1. Analysis of the nucleotide sequence showed an open reading frame of 1653 bp, which was called the citA gene. The citA gene codes for a polypeptide of 551 amino acids.

## SEQUENCE PROTOCOL

&lt;110&gt; Degussa-Hüls AG

5 &lt;120&gt; New nucleotide sequences which code for the citA gene

&lt;130&gt; 000173 BT

&lt;140&gt;

10 &lt;141&gt;

&lt;160&gt; 3

15 &lt;170&gt; PatentIn Ver. 2.1

&lt;210&gt; 1

&lt;211&gt; 2055

&lt;212&gt; DNA

20 &lt;213&gt; Corynebacterium glutamicum

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (201)..(1853)

&lt;223&gt; citA gene

25 &lt;400&gt; 1

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30 ggtggcgcggt gtcacgcaca gaagtgtttg gtgcattgcc tgaggtagtg cgcaaaataa 120

gacttttgtg cattatgatc agaattgttg gcctgggact tcgcttcacg ctctgctgat 180

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1 5 10

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Lys Glu Val Asp Ile Ile Arg Phe Ala Thr Arg Ile Leu Val Ile Gln  
15 20 25

40 gtg gct acc gtc gcg ttg gtg gta gct att tgc acc gga att ttc gca 329

Val Ala Thr Val Ala Leu Val Val Ala Ile Cys Thr Gly Ile Phe Ala  
30 35 40

45 gtt ttg atg atg gat cag atg aaa act gag gcc gag cac aca gcg ctg 377

Val Leu Met Met Asp Gln Met Lys Thr Glu Ala Glu His Thr Ala Leu  
45 50 55

50 tcc atc gga cgt tcg gtg gca tcc aac ccg cag atc cgc gag gaa gta 425

Ser Ile Gly Arg Ser Val Ala Ser Asn Pro Gln Ile Arg Glu Glu Val  
60 65 70 75

55 gcg ctt gat act caa aca gga gca aac cca tcg gcc gaa gaa tta gcc 473

Ala Leu Asp Thr Gln Thr Gly Ala Asn Pro Ser Ala Glu Glu Leu Ala  
80 85 90

gat gga gat atc caa gcg gtt gca cag gcg gcc aat gaa cgc act gga 521

Asp Gly Asp Ile Gln Ala Val Ala Gln Ala Ala Asn Glu Arg Thr Gly  
95 100 105



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	110 115 120	
5	cca gat gag gaa cgt ctg ggg gag cag gtg agc act agc ttt gag gct	617
	Pro Asp Glu Glu Arg Leu Gly Glu Gln Val Ser Thr Ser Phe Glu Ala	
	125 130 135	
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	Ala Met Arg Gly Glu Glu Thr Met Ala Trp Glu Thr Gly Thr Leu Gly	
	140 145 150 155	
15	gcg tcc gcg cga gca aaa gtg cct atc ttt gcg ccg gat tct agt gtt	713
	Ala Ser Ala Arg Ala Lys Val Pro Ile Phe Ala Pro Asp Ser Ser Val	
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	Pro Val Gly Glu Val Ser Val Gly Phe Glu Arg Asp Ser Val Tyr Ser	
	175 180 185	
25	cgc ctg ccc atg ttc ctg gcc gcc ctt gct ctt att tct gtg ttg gga	809
	Arg Leu Pro Met Phe Leu Ala Ala Leu Ala Leu Ile Ser Val Leu Gly	
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30	atc ctt atc ggc gtg ggt gta gcc atg ggc atg cga cgc cgt tgg gaa	857
	Ile Leu Ile Gly Val Gly Val Ala Met Gly Met Arg Arg Arg Trp Glu	
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55	gtt gcc cat aac ggc agg atc ctg tat ctg gat ttc cac ccc gtg cgc	1145
	Val Ala His Asn Gly Arg Ile Leu Tyr Leu Asp Phe His Pro Val Arg	
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55	cgt ggg gat caa gat tta ggc tac gtg gta acc atc cgc gat cgt acc	1193
	Arg Gly Asp Gln Asp Leu Gly Tyr Val Val Thr Ile Arg Asp Arg Thr	
	320 325 330	
	gac atc att gaa ctg agt gaa cgc ctg gac tct gtg cgc acc atg acc	1241
	Asp Ile Ile Glu Leu Ser Glu Arg Leu Asp Ser Val Arg Thr Met Thr	

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10	gca aca ggg ctt atc gac gcc ggc cgc gtc cac gac gcg gca gag ttt Ala Thr Gly Leu Ile Asp Ala Gly Arg Val His Asp Ala Ala Glu Phe 365	370	375	1337
15	cta ggc gat ata tcc cgc aac ggg gga cag tca cat cca ttg atc gga Leu Gly Asp Ile Ser Arg Asn Gly Gly Gln Ser His Pro Leu Ile Gly 380	385	390	1385
20	tca gcg cac ctc aat gaa gca ttt ttg agc tca ttt tta agt act gct Ser Ala His Leu Asn Glu Ala Phe Leu Ser Ser Phe Leu Ser Thr Ala 400	405	410	1433
25	tct att tcg gca tct gaa aag ggc gtt agt ctg cgc atc aac tct gac Ser Ile Ser Ala Ser Glu Lys Gly Val Ser Leu Arg Ile Asn Ser Asp 415	420	425	1481
30	acg ctc atc ctt ggc act gtt aaa gat cca gaa gat gta gca acc att Thr Leu Ile Leu Gly Thr Val Lys Asp Pro Glu Asp Val Ala Thr Ile 430	435	440	1529
35	ttg ggt aat tta atc aac aat gcc atc gac gcc gcg gtg gca ggt gaa Leu Gly Asn Leu Ile Asn Asn Ala Ile Asp Ala Val Ala Gly Glu 445	450	455	1577
40	gcc cca cgg tgg att gag ctt acg ttg atg gat gat gcc gat acg ctg Ala Pro Arg Trp Ile Glu Leu Thr Leu Met Asp Asp Ala Asp Thr Leu 460	465	470	1625
45	gtc att tct gtt gca gat tct ggt cct gga atc cca gag ggc gtg gat Val Ile Ser Val Ala Asp Ser Gly Pro Gly Ile Pro Glu Gly Val Asp 480	485	490	1673
50	gta ttt gcc aca gcc acc cag ata gga gac tct gaa gat aat gaa cgc Val Phe Ala Thr Ala Thr Gln Ile Gly Asp Ser Glu Asp Asn Glu Arg 495	500	505	1721
55	acc cac ggg cat ggc att ggt cta aaa ctg tgc cgg gct ttg gct aga Thr His Gly His Gly Ile Gly Leu Lys Leu Cys Arg Ala Leu Ala Arg 510	515	520	1769
60	tca cat ggt ggc gat gtc tgg gtg att gat aga gga acc gaa gat ggc Ser His Gly Gly Asp Val Trp Val Ile Asp Arg Gly Thr Glu Asp Gly 525	530	535	1817
65	gct gta ttt gga gtg aaa cta ccg gga gta atg gag taatggatca Ala Val Phe Gly Val Lys Leu Pro Gly Val Met Glu 540	545	550	1863
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2055

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 15 20 25 30  
 Leu Val Val Ala Ile Cys Thr Gly Ile Phe Ala Val Leu Met Met Asp  
 35 40 45  
 20 Gln Met Lys Thr Glu Ala Glu His Thr Ala Leu Ser Ile Gly Arg Ser  
 50 55 60  
 Val Ala Ser Asn Pro Gln Ile Arg Glu Glu Val Ala Leu Asp Thr Gln  
 65 70 75 80  
 25 Thr Gly Ala Asn Pro Ser Ala Glu Glu Leu Ala Asp Gly Asp Ile Gln  
 85 90 95  
 Ala Val Ala Gln Ala Ala Asn Glu Arg Thr Gly Ala Leu Phe Val Val  
 30 100 105 110  
 Ile Thr Asp Gly Leu Gly Ile Arg Leu Ser His Pro Asp Glu Glu Arg  
 115 120 125  
 35 Leu Gly Glu Gln Val Ser Thr Ser Phe Glu Ala Ala Met Arg Gly Glu  
 130 135 140  
 Glu Thr Met Ala Trp Glu Thr Gly Thr Leu Gly Ala Ser Ala Arg Ala  
 145 150 155 160  
 40 Lys Val Pro Ile Phe Ala Pro Asp Ser Ser Val Pro Val Gly Glu Val  
 165 170 175  
 Ser Val Gly Phe Glu Arg Asp Ser Val Tyr Ser Arg Leu Pro Met Phe  
 45 180 185 190  
 Leu Ala Ala Leu Ala Leu Ile Ser Val Leu Gly Ile Leu Ile Gly Val  
 195 200 205  
 50 Gly Val Ala Met Gly Met Arg Arg Arg Trp Glu Arg Val Thr Leu Gly  
 210 215 220  
 Leu Gln Pro Glu Glu Leu Val Thr Leu Val Gln Asn Gln Thr Ala Val  
 225 230 235 240  
 55 Ile Asp Gly Ile Asp Glu Gly Val Leu Ala Leu Ser Pro Asn Gly Thr  
 245 250 255  
 Ile Gly Val His Asn Glu Gln Ala Gln Ser Met Ile Gly Ala Gly Pro

	260	265	270
	Met Ser Gly Arg Thr Leu Lys Glu Leu Gly Leu Asp Leu Gly Leu Asp		
	275	280	285
5	Gly Val Val Leu His Gly Gln His Pro Glu Thr Val Ala His Asn Gly		
	290	295	300
	Arg Ile Leu Tyr Leu Asp Phe His Pro Val Arg Arg Gly Asp Gln Asp		
10	305	310	315 320
	Leu Gly Tyr Val Val Thr Ile Arg Asp Arg Thr Asp Ile Ile Glu Leu		
	325	330	335
15	Ser Glu Arg Leu Asp Ser Val Arg Thr Met Thr His Ala Leu Arg Ala		
	340	345	350
	Gln Arg His Glu Phe Ala Asn Arg Ile His Thr Ala Thr Gly Leu Ile		
20	355	360	365
	Asp Ala Gly Arg Val His Asp Ala Ala Glu Phe Leu Gly Asp Ile Ser		
	370	375	380
	Arg Asn Gly Gly Gln Ser His Pro Leu Ile Gly Ser Ala His Leu Asn		
25	385	390	395 400
	Glu Ala Phe Leu Ser Ser Phe Leu Ser Thr Ala Ser Ile Ser Ala Ser		
	405	410	415
30	Glu Lys Gly Val Ser Leu Arg Ile Asn Ser Asp Thr Leu Ile Leu Gly		
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	Thr Val Lys Asp Pro Glu Asp Val Ala Thr Ile Leu Gly Asn Leu Ile		
35	435	440	445
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	Glu Leu Thr Leu Met Asp Asp Ala Asp Thr Leu Val Ile Ser Val Ala		
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	Asp Ser Gly Pro Gly Ile Pro Glu Gly Val Asp Val Phe Ala Thr Ala		
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45	Thr Gln Ile Gly Asp Ser Glu Asp Asn Glu Arg Thr His Gly His Gly		
	500	505	510
	Ile Gly Leu Lys Leu Cys Arg Ala Leu Ala Arg Ser His Gly Gly Asp		
50	515	520	525
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	Lys Leu Pro Gly Val Met Glu		
55	545	550	

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 tgacccttgt gcaaaatcag actgcagtca tcgatggcat tgatgagggc gtgctggcgc 240  
 tgagcccaaa cggaacaatt ggggtgcata atgagcaggc gcaatccatg attggtgcag 300  
 15 gtcctatgag tggcaggacg ttgaaagaac tagggcttga cctgggtctt gatggcgttg 360  
 tattgcatgg tcagcatccg gaaaccgttg ccataacgg caggatcctc tatctggatt 420  
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 c 481

20

## Patent claims

1. An isolated polynucleotide from coryneform bacteria, comprising a polynucleotide sequence which codes for the citA gene, chosen from the group consisting of
  - 5 a) polynucleotide which is identical to the extent of at least 70 % to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 2,
  - 10 b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least to 70% to the amino acid sequence of SEQ ID No. 2,
  - c) polynucleotide which is complementary to the polynucleotides of a) or b), and
  - 15 d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequence of a), b) or c),the polypeptide preferably having the activity of the sensor kinase CitA.
- 20 2. A polynucleotide as claimed in claim 1, wherein the polynucleotide is a preferably recombinant DNA which is capable of replication in coryneform bacteria.
3. A polynucleotide as claimed in claim 1, wherein the polynucleotide is an RNA.
- 25 4. A polynucleotide as claimed in claim 2, comprising the nucleic acid sequence as shown in SEQ ID No. 1.
5. A DNA as claimed in claim 2 which is capable of replication, comprising

- (i) the nucleotide sequence shown in SEQ ID no. 1,  
or
  - (ii) at least one sequence which corresponds to  
sequence (i) within the range of the  
5 degeneration of the genetic code, or
  - (iii) at least one sequence which hybridizes with  
the sequences complementary to sequences (i)  
or (ii), and optionally
  - (iv) sense mutations of neutral function in (i).
- 10 6. A process as claimed in claim 5,  
w h e r e i n  
the hybridization is carried out under a stringency  
corresponding to at most 2x SSC.
- 15 7. A polynucleotide sequence as claimed in claim 2, which  
codes for a polypeptide which comprises the amino acid  
sequence shown in SEQ ID No. 2.
8. A coryneform bacterium, in which the *citA* gene is  
attenuated, preferably eliminated, in particular by  
deletion.
- 20 9. A process for the preparation of L-amino acids, in  
particular L-lysine,  
w h e r e i n  
it comprises carrying out the following steps,
- 25 a) fermentation of the coryneform bacteria which  
produced the desired L-amino acid and in which at  
least the *citA* gene is attenuated,
- b) concentration of the desired product in the  
medium or in the cells of the bacteria and
- c) isolation of the L-amino acid.
- 30 10. A process as claimed in claim 9,  
w h e r e i n

bacteria in which further genes of the biosynthesis pathway of the desired L-amino acid are additionally enhanced are employed.

11. A process as claimed in claim 9,  
5       w h e r e i n  
      bacteria in which the metabolic pathways which reduce the formation of the desired L-amino acid are at least partly eliminated are employed.
12. A process as claimed in claim 9,  
10       w h e r e i n  
      expression of the polynucleotide(s) which codes (code) for the citA gene is reduced, in particular eliminated.
13. A process as claimed in claim 9,  
15       w h e r e i n  
      the regulatory (or catalytic) properties of the polypeptide for which the polynucleotide citA codes are decreased.
14. A process as claimed in claim 9,  
20       w h e r e i n  
      for the preparation of L-amino acids, in particular L-lysine, bacteria in which at the same time one or more of the genes chosen from the group consisting of
- 14.1       the dapA gene which codes for  
25       dihydrodipicolinate synthase,
- 14.2 the gap gene which codes for glyceraldehyde 3-phosphate dehydrogenase
- 14.3 the zwf gene which codes for glucose 6-phosphate dehydrogenase,
- 30       14.4 the pyc gene which codes for pyruvate carboxylase,



- 14.5 the lysE gene which codes for lysine export,
- 14.6 the lysC gene which codes for a feed back  
resistant aspartate kinase,
- 14.7 the zwal gene which codes for the Zwal protein
- 5 is or are enhanced, preferably over-expressed, are  
fermented.
15. A process as claimed in claim 8,  
w h e r e i n  
at the same time one or more of the genes chosen from  
10 the group consisting of:
- 15.1 the pck gene which codes for phosphoenol pyruvate  
carboxykinase,
- 15.2 the pgi gene which codes for glucose 6-phosphate  
isomerase,
- 15 15.3 the poxB gene which codes for pyruvate oxidase
- 15.4 the zwa2 gene which codes for the Zwa2 protein  
is or are attenuated.
16. A process as claimed in one or more of the preceding  
claims,  
20 w h e r e i n  
microorganisms of the genus Corynebacterium glutamicum  
are employed.
17. A process for discovering RNA, cDNA and DNA in order  
to isolate nucleic acids or polynucleotides or genes  
25 which code for sensor kinase Cita or have a high  
similarity with the sequence of the citA gene,  
w h e r e i n  
it comprises employing the polynucleotides comprising

the sequences according to claims 1 to 4 as hybridization probes.

**New nucleotide sequences which code for the citA gene**

## Abstract

The invention relates to isolated polynucleotides comprising a polynucleotide sequence chosen from the group  
5 consisting of

- a) polynucleotide which is identical to the extent of at least 70 % to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 2,
- 10 b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70 % to the amino acid sequence of SEQ ID No. 2,
- c) polynucleotide which is complementary to the  
15 polynucleotides of a) or b), and
- d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequence of a), b) or c),

and a process for the fermentative preparation of L-amino  
20 acids using coryneform bacteria in which at least the citA gene is present in attenuated form, and the use of polynucleotides which comprise the sequences according to the invention as hybridization probes.